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The paternal-sex-ratio (PSR) chromosome in natural populations of *Nasonia* (Hymenoptera: Chalcidoidea)

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sex-ratio distorters.

Abstract

Selfish genetic elements may be important in promoting evolutionary change. Paternal sex ratio (PSR) is a selfish B chromosome that causes all-male families in the haplodiploid parasitic wasp *Nasonia vitripennis*, by inducing paternal genome loss in fertilized eggs. The natural distribution and frequency of this chromosome in North American populations of *N. vitripennis* was investigated using a combination of phenotypic and molecular assays. Sampling throughout North America failed to recover PSR except from populations in the Great Basin area of western North America. Extensive sampling of Great Basin populations revealed PSR in frequencies ranging from 0 to 6% at different collection sites, and extended its distribution to Idaho and Wyoming. Intensive sampling in upstate New York did not detect the chromosome. Frequencies of the maternal-sex ratio distorter (MSR), son killer (SK) and virgin females ranged from 0 to 12%. Paternal sex ratio may be restricted to the Great Basin because its spread is hampered by geographical barriers, or because populations in other areas are not conducive to PSR maintenance. However, it cannot be ruled out that PSR occurs in other regions at very low frequencies. The apparent limited distribution and low frequency of PSR suggest that it will have relatively little impact on genome evolution in *Nasonia*.

Introduction

The idea that genetic elements can be 'parasitic' or 'selfish' is now widely accepted (Östergren, 1945; Dawkins, 1976; Cosmides & Tooby, 1981). Selfish genetic elements are defined as genetic elements that enhance their own transmission and can be neutral or detrimental to their hosts (Werren *et al.*, 1988). Examples include supernumerary (B) chromosomes, meiotic drive chromosomes, transposable elements and cytoplasmically inherited elements. Presence of selfish genetic elements in the genome can lead to 'genomic conflict' (Cosmides & Tooby, 1981) and it has been proposed that such conflict may be a 'motor' for genome evolution (Eberhard, 1980; Cosmides & Tooby, 1981; Werren *et al.*, 1988; Hurst, 1992; Hurst *et al.*, 1996).

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Paternal sex ratio (PSR) is a supernumerary (or B) chromosome found in the parasitic wasp, *Nasonia vitripennis*. It is an extreme example of selfish (or parasitic) DNA. Although PSR bearing males produce functional sperm that fertilizes eggs, the paternal chromosomes (except PSR) fail to condense properly in the first mitotic division, and are eventually lost (Werren *et al.*, 1987; Reed & Werren, 1995). The effect of PSR action is to convert diploid eggs, which would have developed into females, into haploid males, thus causing the production of all-male families (Werren *et al.*, 1981; Nur *et al.*, 1988; Werren, 1991). These males carry the PSR chromosome, which will again destroy the male's sperm chromosomes following fertilization. Thus, PSR 'jumps' from one set of autosomes to another each generation, totally eliminating the autosomal genome of males with which it is associated, making PSR the most extreme example of selfish DNA so far described in any organism (Werren, 1991).

In addition to PSR, two other sex-ratio distorters have been found in *N. vitripennis*. Maternal sex ratio (MSR) is

cytoplasmically transmitted and causes carrier females to produce nearly all-female progenies (Skinner, 1982). Its aetiology is still unknown. Son killer (SK) is a maternally transmitted bacterium (Huger *et al.*, 1985; Skinner, 1985; Werren *et al.*, 1986), named *Arsenophonus nasoniae* (Gherina *et al.*, 1991) that kills male eggs and therefore causes all- or nearly all-female broods.

Two sibling species *N. vitripennis* occur in North America. *N. giraulti* is restricted to the east and *N. longicornis* to the west of the continent (Darling & Werren, 1990). Both species are reproductively isolated from each other and from *N. vitripennis* through infection by different strains of *Wolbachia* bacteria which cause cytoplasmic incompatibility in interspecies crosses (Breeuwer & Werren, 1995; Bordenstein & Werren, 1998).

The PSR chromosome was originally detected during selection experiments for sex ratio using natural isolates of *N. vitripennis* (Werren *et al.*, 1981). Skinner (1983) subsequently found PSR at frequencies up to 11% in natural populations around Salt Lake City, Utah. Theoretical and experimental studies have shown that PSR frequency should be strongly dependent upon population structure and the egg fertilization rate (fertilized eggs develop into females when fertilized by normal sperm but into PSR males when fertilized by PSR-bearing sperm) (Beukeboom & Werren, 1992; Werren & Beukeboom, 1993). The PSR frequency is also expected to be influenced by the presence of MSR, because this cytoplasmic factor induces a higher than normal frequency of egg fertilization (Skinner, 1982). Thus, PSR presents an interesting system for investigation of the codynamics of interacting sex-ratio distorters and the role of subdivided population structure in distorter dynamics. However, little is known about the frequency and distribution of PSR in natural populations. In this paper, we report results of studies on the distribution and frequency of PSR and related sex-ratio distorters in all three *Nasonia* species. We specifically address whether associations between sex-ratio distorters occur, as predicted by theory. In addition, we experimentally test whether PSR can be maintained in *N. longicornis* and *N. giraulti*.

Materials and methods

Field collections of *Nasonia*

Nasonia wasps parasitize pupae of blowflies and fleshflies (Whiting, 1967; Darling & Werren, 1990). They can therefore be found where these flies occur. Protocalliphorid blowflies occur at bird nests, where the larvae are blood-feeding ectoparasites. All three species of *Nasonia* parasitize the pupae of these flies found in bird nests. *Nasonia vitripennis* also parasitizes calliphorid, sarcophagid, and other flies occurring at carcasses, and other sites. We therefore collected wasps from three sources, bird nests, carcasses and baits (described below). Two basic methods of wasp collection were used; either (a) adult

females arriving at a site were collected (Adult lines) or (b) host pupae were collected from the field and emerging wasps were collected for analysis (Emergence lines). For Emergence lines, collections of pupae from a single site (a nest, bait or carcass) were placed in a ziplock bag, or for nests the entire nest was often collected, always after the young birds had fledged. Emerging wasps were permitted to mate within the bag and then a sample of females was collected for analysis. The important distinction of the two approaches is that Adult-line females presumably represent a 'random' sample of adult females within a local population, whereas emergence-line females emerge from a specific patch of hosts and therefore can be closely related, depending upon the number of ovipositing female parents (foundresses) at the site. Details of collection methods are provided below.

Sampling areas

Field sampling of *Nasonia* was conducted from 1986–1991. The first few years focused on upstate New York. From 1986–1989 spot sampling throughout the United States was accomplished using bird nest material shipped to the laboratory by members of the North American Bluebird Society. Sampling of Utah populations began in 1988 and continued until 1991. Studies were extended to parts of Idaho, Nevada and Wyoming in 1990 and 1991. Adult lines were collected from Utah in 1990 and 1991 and from Idaho, Nevada and Wyoming in 1991.

Bird nests

Bird nests were collected after fledging of the birds, placed in closed zip-lock bags and taken into the laboratory. Nests were observed daily for wasp emergences. As soon as the wasps were discovered, the nest was put into a plastic tub with two holes on top, over which a vial was placed. Wasps that migrated into the vial were collected and a subset (usually 1 of 5 or 10) was individually provided with hosts for oviposition. In addition to our own collections, members of the North American Bluebird Society provided nests for these studies.

Carcasses

Arriving adult wasps and fly pupae were collected from carcasses found in the study areas. These were found mainly along the sides of roads, and included a variety of animals (primarily porcupines, rabbits, deer, and fish). In most cases, the carcass had been observed for several days to determine the progression of fly oviposition and development. In other cases, the fly pupae under the carcass could be seen to be young (due to colour) and fly pupae with fly- and wasp-emergence holes were not observed. For these reasons, we were confident that the adult wasps collected from the patches represented arriving adults rather than emerging offspring from the

patch. In cases where emergences could have occurred, Adult-line females were not collected from the site.

Baits

Mesh-screen bags containing laboratory raised *Sarcophaga bullata* pupae and a liver remains previously fed to fly larvae in the laboratory were used as baits. They were usually placed underneath carcasses (road kills), near bird nests, in barns, or out in the open field or woods and collected several hours to several days (up to 5) later. Adult wasps found in the mesh bags were collected from baits and set as Adult lines. Because the generation time of the wasps in the field is 14 + days (depending upon temperature) wasps had not yet emerged from the hosts, these were known to be arriving at the baits. In some cases, parasitized pupae from the baits were also sent to the laboratory for analysis (Emergence lines).

Processing of samples

Each sample was given a unique coding based upon the type of collection (Adult or Emergence), state of collection, whether bait, carcass or nest, and a number identifying the patch and year of collection. Specific-collection localities have been described in more detail in Beukeboom (1992).

Adult-line females were given two *S. bullata* hosts and then shipped back to the laboratory for analysis. Emerging progeny were scored for sex ratio and species. A sample was then collected for processing to determine presence of PSR (among high-male and all-male families), MSR and SK using molecular or phenotypic assays described below. Emergence-line females were also given two hosts and the progeny were processed in the same fashion as Adult-line females.

Assays for PSR, MSR, SK and virgin females

Both phenotypic and molecular assays were used to detect the presence of PSR. The phenotypic assay relies on the fact that the PSR trait is transmitted to 'sons'. All-male and high-male families in the F₁-generation of Adult and Emergence females were tested. In the first years of the study, a sample of 4–5 males was individually mated to virgin females of a standard laboratory strain (LabII). Sex ratio among resulting offspring was recorded. Sex ratios of 90–100% males were scored as potentially PSR and retested on another generation. Families showing inheritance of all-male or high-male traits were scored as PSR. High-male sex ratio families were included, because PSR males sometimes do not transmit the chromosome to every fertilized egg, resulting in daughter production (Beukeboom & Werren, 1993). In 1990 and 1991, the high and all-male progenies were further tested for PSR using the molecular assay described in Beukeboom & Werren (1992). Briefly, groups of five males from a

family were homogenized and their DNA hybridized to a radioactively labelled probe of a PSR-specific repetitive sequence in a dot-blot assay. All-male families that did not probe positive for PSR were attributed to unmated (virgin) females.

Assays for SK females are described in detail in Balas *et al.* (1996). Briefly, either the original female or her progeny were homogenized and probed for SK using radiolabelled total genomic DNA from a SK strain. Isolation of SK bacteria from infected females confirmed the reliability of the molecular assay.

Assays for the MSR trait were less thorough. Among females producing all-female progeny, those found not to harbour SK are presumed to be MSR. All-female progeny are unusual among 'normal females' under the conditions reported here (two hosts), however, this was not specifically quantified. The assay is more likely to under-report MSR females because these females can produce some males under these conditions, although typically at frequencies much lower than non-MSR females. Emergence lines were not systematically screened for MSR and SK and we therefore report frequencies of all-female progenies. Adult lines that produced all-female progeny were screened for SK with the molecular assay. In addition, for many samples, four female progeny were taken and given four hosts to determine the sex ratio – the line produced under this situation. *Nasonia* females are known to elevate proportion males when ovipositing in a group, except that MSR females and SK females produce much more female biased sex ratios under this circumstance. Sex ratios of 15% male or less were attributed to MSR once the possibility of SK was ruled out. In some cases, the original Adult-line female was assayed for SK, rather than her progeny.

PSR expression in *N. giraulti* and *N. longicornis* and *Wolbachia*-free wasps

Experiments were undertaken to determine whether the PSR chromosome from *N. vitripennis* is capable of expression (inducing all-male families) and transmission in the two sibling species, *N. giraulti* and *N. longicornis*, or in *N. vitripennis* free of the bacterial (*Wolbachia*) endosymbionts (Breeuwer & Werren, 1990). Before PSR could be introgressed from *N. vitripennis* to any of the sibling species, cytoplasmic incompatibility barriers between the species had to be overcome (Breeuwer & Werren, 1990, 1995). This was accomplished by curing a PSR (LabII) strain of its endosymbionts by feeding PSR-mated females with tetracycline (1 mg mL⁻¹). Asymbiont *N. vitripennis* males are compatible with asymbiont and symbiont females from each species. The asymbiont PSR (LabII) males were first mated with AsymC females of *N. vitripennis*. AsymC is a LabII strain permanently cured of its micro-organisms (Breeuwer & Werren, 1990). Resulting PSR (AsymC) males were

then crossed to both symbiont (RV2) and asymbiont (RV2T where T stands for tetracycline treated) lines of *N. giraulti*, as well as symbiont (IV14) and asymbiont (IV14T) lines of *N. longicornis*. Because some interspecific crosses are hampered by behavioural differences in courtship, copulations were confirmed by observation. Sex ratios of progenies were scored and all-male and high-male families subsequently screened using the molecular assay to confirm the presence of PSR. Within each lineage, expression and transmission of PSR was examined over three generations by previously described crosses between virgin females and PSR carrier males.

Statistical analysis

Two estimates were used for the frequencies of PSR, MSR, SK and virgin females. The first is simply the number of carrier females divided by the total number of females tested ('overall frequency'). The second method first estimated the frequency per patch. A patch consists of a collection site, i.e. a nest, a bait or a carcass. Frequencies were subsequently arcsin transformed and an average plus standard deviation was estimated over all patches in each state ('average frequency'). Note that the number of females per patch could differ by a factor 100 but that all patch sizes with five or more females were counted equally. Moreover, this second method was only applied to Adult lines, because frequencies among Emergence lines can be strongly affected by mating among offspring in the collection containers and therefore do not fully reflect the natural situation. For states in which PSR was not found, 95 and 99% confidence upper limits were estimated using the binomial distribution. This was only useful for states from which a large number of lines had been tested.

Associations between two sex-ratio distorters (including virginity) were tested using randomization tests (Gotelli, 1996). This was only done for the Adult lines as the Emergence lines did not yield enough data. For example, we tested the hypothesis that PSR and MSR occurred more frequently in the same patch than expected, based on random distribution. We used three variables that were based on the actual number of collected females: (1) the number females in each sampled patch (2) the number of females that were PSR-mated, and (3) the number of MSR females. We then randomly seeded the observed number of PSR and MSR females across patches by keeping the number of females per patch fixed (as collected). This was repeated 1000 times using a customized random model simulation programme written in Java. We then used the simulated frequencies as an estimate (*P*-value) for how frequently PSR and MSR would have been found together in a patch under random distribution and compared this with the observed within patch association.

Results

PSR and other sex ratio distorters in *N. vitripennis*

Emergence samples

A total of 2891 Emergence-line females were sampled from 185 nests in 15 states of North America during the years 1988–91 (Fig. 1). Most nests came from New York (102), Minnesota (27) and Utah (16). In addition, 325 wasps from 89 nests were sampled from New York in 1986 and 1987. These earlier collected Emergence lines are not shown in Fig. 1 because they were not systematically tested for all-female progenies.

Among Emergence lines, PSR was only found in nests from Utah, at a frequency of 2.5%. All-female progenies were found in several states at low frequencies, but up to 13.3% in Minnesota (all states pooled 3.2%). These all-female families were not systematically tested for MSR or SK. The results indicate that PSR was rare or absent from wasps parasitizing birdnest blowflies throughout North America. For New York, the estimated upper 95 and 99% limits of PSR are 1.6 and 2.3%, respectively ($n = 191$ nests). If one uses individual wasps instead of nests, the frequencies are 0.23 and 0.15%, respectively ($n = 1966$ wasps). In Minnesota, the only other state from which many nests had been collected, the 95 and 99% probabilities of not finding PSR among 557 wasps yielded upper limits for PSR frequency of 0.53 and 0.82%, respectively.

PSR was previously known to occur in populations of *N. vitripennis* collected in northern Utah (Werren *et al.*, 1981; Skinner, 1982). The area lies within the Great Basin, a large region including parts of Utah, Nevada, Wyoming, Idaho and Washington with drainage into the Great Salt Lake. Great Basin populations in western North America were therefore extensively sampled for PSR in 1990 and 1991 by putting out baits and sampling from carcasses. The results of these studies are shown in Table 1. We tested 924 wasps emerging from 56 baits (Table 1a) and 298 wasps (Table 1b) from 26 carcasses. This yielded PSR among 12 females from a total of 5 patches corresponding to low frequencies in Utah (1.3 and 0.5%), Idaho (0.3%) and Wyoming (6.1%). The overall PSR frequency estimated by pooling all states was 1.0% ($n = 924$ wasps) for baits and 1.0% ($n = 298$) for carcasses.

All-female frequencies ranged from 0% in Nevada to 18.2% in Wyoming. The overall frequency estimated by pooling all states was 6.3% ($n = 924$) for baits and 10.4% ($n = 298$) for carcasses.

Adult samples

A total of 488 field adults were collected from 77 patches in 1990 and 1991 from the Great Basin area (Utah, Wyoming, Nevada and Idaho). They were tested for PSR, MSR, SK and virginity (Table 2). Paternal-sex ratio was found in six females at 2 patches in 1990 in Utah and one

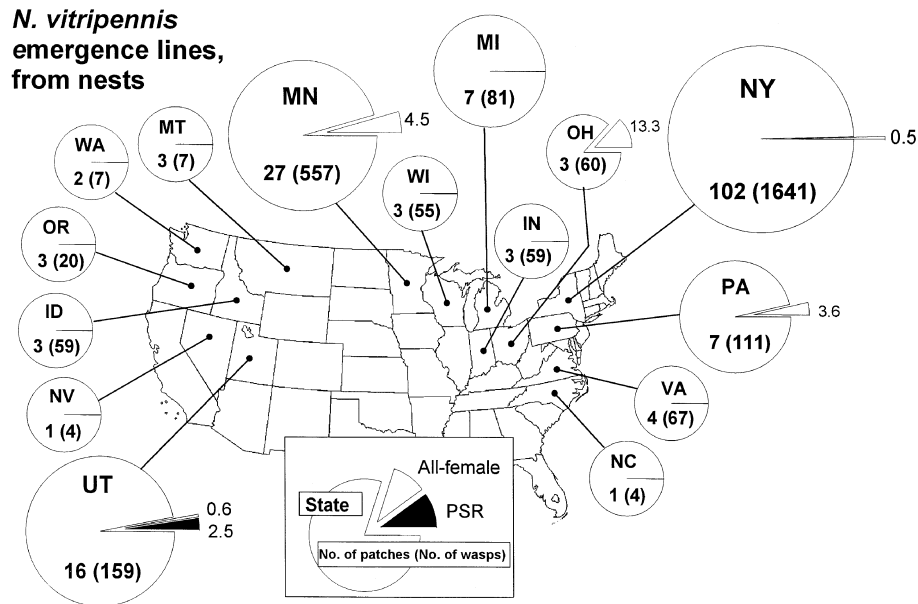


Fig. 1 Frequencies (in percentages) of PSR (black slice) and all-female progenies (white slice) of *N. vitripennis* Emergence lines collected from nests in North America. State abbreviations are shown in the upper part of each pie diagram and number of patches and wasps (in parentheses) in the lower part. Pie sizes reflect the number of patches and wasps that were tested for each state.

Table 1 Frequencies (in percentages) of PSR and all-female progenies among *N. vitripennis* Emergence lines from (a) baits and (b) carcasses of the Great Basin area.

State	Number of patches sampled	Number of females tested	Number of PSR-mated females (patches)	PSR-mated females (%)	Number of all-female progenies (patches)	All-female progenies (%)
(a)						
Idaho	18	307	1 (1)	0.3	22 (7)	7.2
Nevada	1	13	0	0	0	0
Utah	37	604	8 (2)	1.3	36 (13)	6.0
ALL	56	924	9 (3)	1.0	58 (20)	6.3
(b)						
Idaho	5	54	0	0	3 (1)	5.6
Utah	20	211	1 (1)	0.5	22 (9)	10.4
Wyoming	1	33	2 (1)	6.1	6 (1)	18.2
ALL	26	298	3 (2)	1.0	31 (11)	10.4

female each at 2 patches in 1991 in Idaho. This yielded overall PSR frequencies of 2.7 and 0.8%, respectively. The total overall frequency of PSR in 1991 pooling all four states was 0.4%. Average frequencies (≥ 5 females per patch) were less than 1%. The overall frequency of MSR over all states in 1991 was 3.3% and ranged from 1.6 to 12.5% per state. The SK frequencies were very similar; the frequency over all states was 2.9% in 1991 (range 0.4–12.5%). Of 40 females producing all-female progenies, 23 (58%) were assigned to MSR after 17 (42%) probed positive to SK. The majority of all-female progenies were not due to PSR and therefore were attributed to virgin females. Overall frequencies of virgin

females were 6.7% in Utah in 1990 and ranged from 0 to 7.3% per state in 1991 (6.3% over all states). Average frequencies (> 5 females per patch) of virgin females were 2–4% (Table 2).

Possible associations between the different sex-ratio distorters were tested by random simulation (see methods). Associations were tested at two different geographical scales, i.e. at the level of individual patches and groups of patches from close vicinity (regions). In no case were significant positive or negative associations detected for any two sex-ratio distorters or virgin females ($P > 0.05$). In many cases, the number of females in each sex-ratio distorter category were very low (see Table 2).

Table 2 Frequencies (in percentages) of PSR, MSR, SK and virgin females among *N. vitripennis* Adult lines from the Great Basin Area.

State + year	No. of patches sampled	No. of females tested	No. of PSR-mated females (patches)	PSR-mated females ¹ (%)	Average per cent +SD of PSR-mated females	No. of MSR females (patches)	MSR females ¹
Utah 1990	27	223	6 (2)	2.7	0.34 + 1.74 (N = 11)	7 (4)	3.1
Idaho 1991	26	246	2 (2)	0.8	0.18 + 0.72 (N = 9)	6 (5)	2.4
Nevada 1991	9	62	0	0	0 (N = 2)	1 (1)	1.6
Utah 1991	14	172	0	0	0 (N = 7)	8 (5)	4.7
Wyoming 1991	1	8	0	0	0 (N = 1)	1 (1)	12.5
ALL 1991	50	488	2 (2)	0.4	0.04 + 0.37 (N = 19)	16 (12)	3.3

State + year	Average % + SD of MSR females ²	No. of SK females (patches)	SK females ¹ (%)	Average % + SD of SK females ²	No. of virgin females (patches)	Virgin females ¹	Average % + SD of virgin females ²
Utah 1990	0.67 + 2.26	19 (10)	8.5	6.91 + 3.50	15 (9)	6.7	4.22 + 3.06
Idaho 1991	0.96 + 3.77	7 (7)	2.8	2.36 + 2.87	18 (9)	7.3	3.99 + 2.56
Nevada 1991	0.86 + 1.71	1 (1)	1.6	0.86 + 1.71	3 (3)	4.8	4.07 + 0.06
Utah 1991	2.35 + 6.22	5 (4)	2.9	0.57 + 0.98	10 (7)	5.8	2.39 + 2.85
Wyoming 1991	12.5	1 (1)	12.5	12.5	0	0	0
ALL 1991	1.73 + 4.23	14 (13)	2.9	1.67 + 2.15	31 (19)	6.3	2.99 + 2.32

¹ Number of carrier females divided by total number of females tested.² Per cent of carrier females per patch averaged over all patches (see methods), sample sizes (N) are shown in the PSR column.

Distribution of PSR

Thus far, PSR had only been reported from the Great Salt Lake area (Skinner, 1983). This study found PSR at 12 localities in the Great Basin area and extended its current known range to approximately 150 × 350 km covering

northern Utah, south-eastern Idaho and western Wyoming (Fig. 2).

Sex-ratio distorters in *N. longicornis* and *N. giraulti*

Emergence samples

A total of 710 *N. longicornis* wasp emergences were tested from 55 nests in five western states (California, Montana, Nevada, Utah and Wyoming) and 372 *N. giraulti* wasps were tested from 62 nests in six north-eastern states (Indiana, Michigan, New York, Ohio, Pennsylvania and Virginia) (Fig. 3). Paternal-sex ratio was not found in either of these two sibling species. Frequency of all-female progenies ranged from 0 to 4.4% in *N. longicornis* (all states pooled 1.5%) and 0–8.0% (all states pooled 4.0%) in *N. giraulti*, which is very similar to *N. vitripennis*. These all-female progenies were not tested for MSR and SK, but SK has previously been found in *N. longicornis* (Balas *et al.*, 1996).

A total of 14 baits from the Great Basin area yielded 215 *N. longicornis* wasps. Again, no PSR was found. All-female progenies occurred at a frequency of 1.9% in Utah (4 of 208 females in 12 patches). A total number of 11 Emergence-line females from one carcass did not yield any PSR or all-female progenies.

Adult samples

Field adults of *N. longicornis* were collected. Paternal-sex ratio was not found among 30 females from 8 patches (3 × Idaho, 2 × Nevada, 3 × Utah). Frequencies of SK over all three states were 6.7% (range 0–17%). One mother of an all-female clutch was not SK and might therefore be MSR, although we have never formally tested for cytoplasmic inheritance of the MSR trait in *N. longicornis*. No virgin females were found.

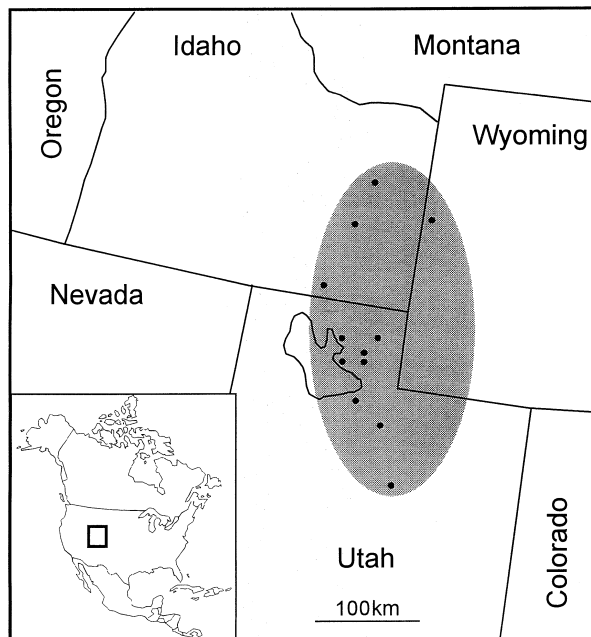


Fig. 2 Current known distribution of PSR restricted to the Great Basin area. Details of collection sites are given in Beukeboom (1992).

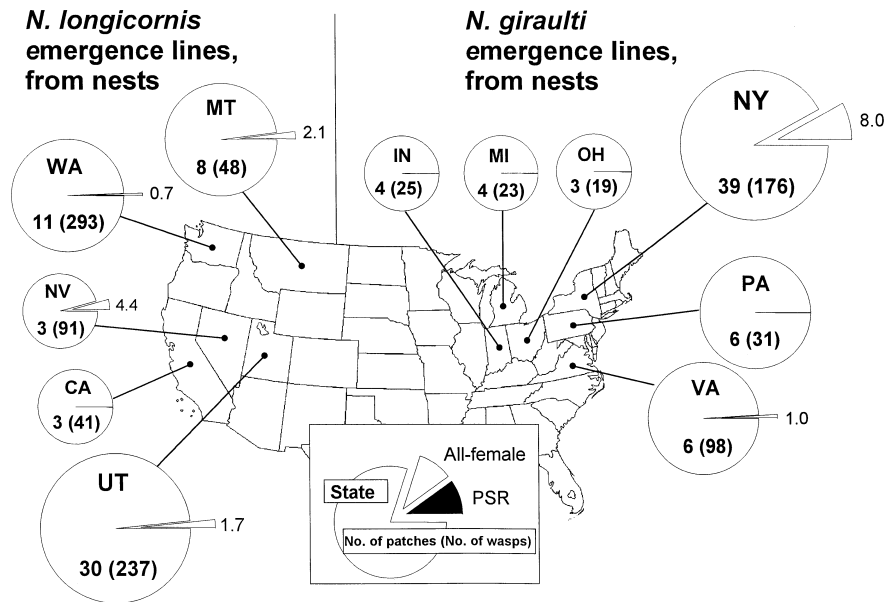


Fig. 3 Frequencies (in percentages) of PSR (black slice) and all-female progenies (white slice) of *N. longicornis* (western states) and *N. giraulti* (eastern states) Emergence lines collected from nests in North America. State abbreviations are shown in the upper part of each pie diagram and number of patches and wasps (in parentheses) in the lower part. Pie sizes reflect the number of patches and wasps that were tested for each state.

Experimental introduction of PSR into *N. longicornis* and *N. giraulti*

Paternal-sex-ratio was successfully maintained for three generations in the asymbiont AsymC strain of *N. vitripennis*, showing that PSR functions normally in the absence of *Wolbachia* bacteria. Experiments were also conducted to determine if PSR could be transmitted and function (i.e. destroy the paternal chromosomes) in the two sibling species of *N. vitripennis*. Introgression of PSR into asymbiont and symbiont strains of *N. giraulti* and *N. longicornis* resulted in the production of all-male families that carried PSR; PSR was subsequently successfully maintained for three generations in both species, showing that PSR action is not restricted to *N. vitripennis* (data in Beukeboom, 1992). Dobson & Tanouye (1996, 1998) independently found that PSR functions normally in *Wolbachia*-free lines of *N. vitripennis* as well as in the other two species.

Discussion

PSR is the most extreme example of selfish DNA found in any organism (Nur *et al.*, 1988; Werren, 1991). The reason for this 'moniker' is that PSR completely destroys the genome of its host each generation, reducing host fitness to zero. If PSR were to become common in natural populations, it could drive such populations to extinction. Some theoretical and experimental studies (Beukeboom & Werren, 1992; Werren & Beukeboom, 1993) suggest that PSR frequency may be constrained by

fertilization rate and population structure. However, codynamics with other sex-ratio elements (such as MSR) could result in high frequency of PSR.

Our results indicate that PSR is not particularly successful or widespread in nature. To date, it has only been found in the Great Basin area in western North America. It is most likely absent in upstate New York, but might be present in other regions that have not been sampled extensively. It is not known whether its current known distribution range extends into adjacent regions, such as Montana, Colorado and Oregon. However, current data suggest a limited distribution in western North America.

Given that *N. vitripennis* occurs world wide, how can we explain such a limited distribution for PSR? The most likely explanation is that PSR has arisen relatively recently in *N. vitripennis* in the Great Basin region of North America, and has not yet dispersed to other parts of the distribution of *N. vitripennis*. This is not inconceivable, given that most of the regions surrounding the Great Basin are desert and/or mountains. *Nasonia* appears to be absent in desert environments based on scattered observations (unpublished results). In addition, dispersal of PSR into low-density populations would be difficult because the chromosome depends on the production of females within local-mating patches for its maintenance (Beukeboom & Werren, 1992; Werren & Beukeboom, 1993). These studies have shown that PSR cannot exist in highly substructured populations consisting of small local mating groups (demes), e.g. smaller than three

foundresses, nor in panmictic populations in which the fertilization proportion is 50%.

Molecular evidence indicates that PSR came into *N. vitripennis* via an interspecies mating with wasps from a closely related genus, *Trichomalopsis* spp. (McAllister & Werren, 1997). One hypothesis is that PSR was generated by chromosome fragmentation during the interspecies hybridization. If this is correct, then the presence of several families of tandem repetitive DNA unique to PSR (Eickbush *et al.*, 1992) must have undergone rapid amplification subsequent to the interspecies hybridization. An alternative hypothesis is that PSR was (or is) an extant B chromosome in *Trichomalopsis* that has moved into *N. vitripennis* by a recent (and presumably rare) intergenus hybridization event (McAllister & Werren, 1997). This latter model could also explain the limited geographical distribution of PSR to western North America, where presumably the horizontal transfer occurred.

Parental-sex-ratio was found at overall frequencies of 0–6% at sampling sites. This is somewhat lower than previously reported values of 11% (Skinner, 1983; see also Werren, 1987), suggesting that PSR may be declining. Frequencies were similar among wasps collected from nests, baits and carcasses which suggest no differences in habitat preferences of PSR-carrier and non-carrier females. Both theoretical and empirical studies indicate that it is difficult for PSR to reach frequencies above 20% unless MSR achieves high frequencies (Werren, 1987; Beukeboom & Werren, 1992; Werren & Beukeboom, 1993). Fertilization proportion and local deme sizes are major determinants in PSR dynamics. The high fertilization proportion of MSR females was shown to greatly increase PSR frequencies and PSR may depend on the presence of MSR for its existence (Beukeboom & Werren, 1992; Werren & Beukeboom, 1993). Nevertheless, the distributions of PSR and MSR were not positively correlated in this study. This may in part be due to the fact that both distorters occurred at low frequencies which limits the strength of the statistical analyses.

Both SK and MSR result in high-female or all-female progenies. Although assays were not performed for SK vs. MSR in the Emergence-line samples, the Adult-line samples showed that somewhat less than 50% of all-female progenies were due to SK in the Great Basin area. The overall SK frequency was 8.5% in 1990 and 2.9% in 1991 which is rather similar to 11% (1990) and 7.6% (1992) reported by Balas *et al.* (1996). The MSR frequencies ranged from 0 to 12% which is similar to that reported by Skinner (1983).

Paternal-sex-ratio was not found in the two sibling species of *N. vitripennis*. *Nasonia vitripennis* occurs microsympatrically with *N. longicornis* in western North America and with *N. giraulti* in eastern North America. Absence of PSR from *N. giraulti* is not surprising because its distribution range is restricted to eastern North America whereas PSR in *N. vitripennis* only occurs in the Great Basin area. However, the distribution range of

N. longicornis clearly overlaps with that of PSR and many *N. longicornis* females have been collected from the Great Basin area. Moreover, in one instance *N. longicornis* females were collected from the same patch from which PSR-mated *N. vitripennis* females were collected, indicating that *N. longicornis* could come in contact with PSR. Furthermore, our laboratory experiments demonstrated that PSR can be successfully maintained in *N. giraulti* and *N. longicornis* showing that PSR action is independent of *Wolbachia* strains (Beukeboom, 1992; Dobson & Tanouye, 1998). Nevertheless, it may be difficult for PSR to be introgressed from *N. vitripennis* to *N. longicornis* in natural populations because of gene flow barriers, due to the presence of interspecies cytoplasmic incompatibility caused by *Wolbachia* (Breeuwer & Werren, 1990). However, it has not been ruled out that PSR occurs in *N. longicornis* at low frequencies.

In conclusion, although PSR is an extreme form of selfish DNA, its frequency in natural populations of *N. vitripennis* is low and its distribution apparently limited to the Great Basin of Western North America. Therefore, effects of PSR on genome evolution in *N. vitripennis* are unlikely to be large.

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